

## Drug candidate affinity for human serum albumin and $\alpha_1$ -acid glycoprotein / Plasma protein binding

### Purpose

Plasma protein binding of drug candidates affects their pharmacokinetic and pharmacodynamic properties. The most important proteins involved in plasma protein binding are human serum albumin (HSA) and  $\alpha_1$ -acid glycoprotein (AGP), tending to bind compounds independently on their structure in distinct binding sides. HSA preferably binds acids, whereas basic or lipophilic compounds usually bind to AGP. A fast and reproducible way to quantitatively study HSA and AGP binding properties of drug candidates is to determine the dissociation constant ( $K_d$ ) for the respective protein using the Transil® technology provided by Sovicell GmbH, Leipzig.

### Assay protocol

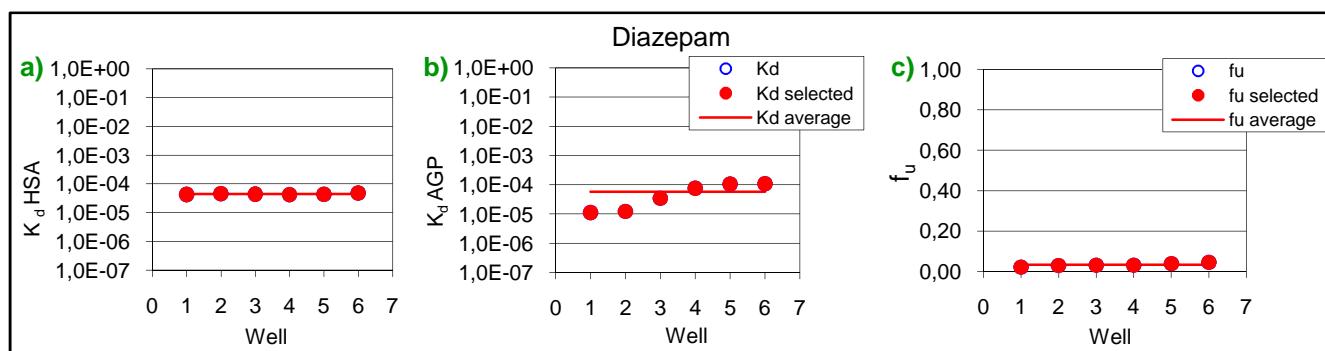
Transil® HSA or AGP binding kits consist of either HSA or AGP immobilized on inert Transil® beads, which are characterized by their low-binding surface. The affinity of drug candidates to bind to HSA or AGP is tested by the addition of a constant amount of test compound to varying amounts of protein bound to beads and using phosphate buffered saline as matrix in a 96-well-plate format. The unbound fraction in the supernatant is quantified by LC/MS after separation by centrifugation and is used to calculate  $K_d$  values. Total plasma protein binding is determined using Transil® Plasma Protein binding kits that consist of HSA and AGP at a physiological ratio.

### Results

For model drugs with high, low and medium protein binding properties (data obtained from SPC of the respective drug),  $K_d$  values for HSA, AGP and PPB. Results are summarized below.

**Table 1:** HSA  $K_d$  values and total plasma protein binding of model drugs

Reference drug	$f_b$ HSA Transil® [%]	CV [%]	$K_d$ HSA	CV [%]	$f_b$ PPB Transil® [%]	CV [%]	% PPB (SPC human)
Carbamazepine	60.3	5.8	3.9.E-04	4.6	62.6	12.9	71%
Diazepam	93.5	1.7	4.2.E-05	14.1	97.0	0.7	98%
Methotrexate	73.6	8.3	2.2.E-04	18.5	85.9	2.2	50%
Metoprolol	20.7	9.2	2.4.E-03	16.3	37.5	10.0	10%
Warfarin	98.1	0.2	1.2.E-05	5.6	98.2	0.2	>99%



**Figure 1** a)  $K_d$  of diazepam for HSA  
b)  $K_d$  of diazepam for AGP  
c) PPB of diazepam

$f_u$  = unbound fraction      PPB = plasma protein binding  
 $f_b$  = bound fraction       $K_d$  = dissociation constant  
 % CV = coefficient of variation  
 SPC = Summary of Product Characteristics

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Please don't hesitate to contact us for a customized quotation

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